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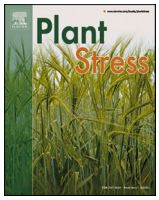

Large scale phenotyping on the effect of heat and cold stress on *Brassica napus* during floral development☆

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ABSTRACT

As a result of climate change temperature extremes are being frequently experienced, which are endangering agricultural production. Oilseed rape (*Brassica napus* L.; OSR) is an important cool-season oil crop grown worldwide; however, extremes of cold and heat are major threats to production that cause considerable yield losses especially at the flowering developmental stage. The aim of this study was to evaluate the physiological performance of diverse oilseed rape genotypes during reproduction under cold and heat stress. 94 diverse genotypes with variable characteristics and temperature tolerances were treated for 3 days under cold (6/4 °C) and heat (35/23 °C) stress, and yield traits were analysed. Phenotypic analysis of these rapeseed genotypes revealed the impact of cold/heat stress on flowering periods, with cold stress having a minimal, non-significant negative effect on final yield. Heat stress reduced seed yield by 1.3 fold ($P = 0.0009$), with a significant effect on numerous parameters collected from the main raceme including, seed weight, number, and seed pod fertility. We have identified 28 genotypes that appear tolerant to heat stress, with 5 maintaining high yield that could be used for breeding of heat-tolerant lines for future climate changes.

1. Background

Oilseed rape (*Brassica napus* L., OSR) ranks as the world's second-largest source of oil crop. It has a high nutritive quality and is currently cultivated on 35 million ha worldwide in 2022 (Zheng and Liu, 2022) and 342,000 ha within the UK (2023; https://www.gov.uk/government/statistics/cereal-and-oilseed-rape-areas-in-england/cereal-and-oilseed-rape-areas-in-england-at-1-june-2023). Improving OSR yield and seed quality to meet market demands is critical, and a better understanding of the environmental interactions is essential. As low erucic acid and glucosinolate varieties were only developed in the 1970s (Lin et al., 2013), there is still significant improvement that can be harnessed from different cultivars/breeding programmes, with 73 % of yield variability attributed to environmental factors (Tetteh et al., 2019). Flowering is extremely susceptible to environmental conditions in most crop species, including OSR (He et al., 2017). Flower production is a critical factor influencing final yield, with most of the mature pods (75 %) forming in the first 14 days in OSR (Tayo and Morgan, 1975); understanding the effect of environmental factors on fertility is essential to help maintain yield. Winter and spring OSR genotypes have been developed, based on vernalisation requirements and growth season, and as a cool-season crop its yield can be reduced on exposure to both low and high temperatures (Singh et al., 2008).

In the UK, winter OSR flower from April-June, with floral meristems forming in March, while spring varieties bloom June-July, with floral meristems forming in May. This means that during flowering these varieties can be subjected to a mean minimum temperature in the UK as low as 2.4 °C/7 °C in March/May, with mean maximum temperatures of 21.2 °C/18.8 °C in June/July, and with highest maximum of 30.2–32.2 °C (Met Office, 2023). Temperatures of 15–20 °C are optimal for growth and development of OSR (Rahaman et al., 2018).

Cold stress, both freezing (<0 °C) and chilling (<10 °C), affects OSR by impairing plant physiology and biochemistry, negatively influencing photosynthesis and respiratory metabolism, increasing oxidation stress

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and ultimately reducing growth. Chilling stress during flowering can induce flower abortion, pollen and ovule infertility, with a breakdown of pollination/fertilisation and seed filling, leading to lower yields (Lardon and Triboi-Blondel, 1995; Thakur et al., 2010). Early morning frosts and cold snaps can damage both vegetative and reproductive OSR organs (Larden and Triboi-Blondel, 1994).

OSR is also extremely sensitive to heat stress, particularly during the reproductive stages (reviewed by Secchi et al., 2023). High temperature stress is one of the most important abiotic stresses affecting plant productivity worldwide and has been studied in various plant species, it is linked to infertility and yield loss (pod number, seeds per pod and seed weight) (Quezada-Martinez et al., 2021; Zhu et al., 2021). Heat stress has a negative impact on plant growth, influencing cell (photosynthetic) membranes, protein synthesis, stability, and degradation, decreased efficiency of photosystem II and photosynthesis, increased production of toxic metabolites, reduced oil synthesis/accumulation and reduced seed filling (Morrison and Stewart, 2002; Kirkegaard et al., 2018; Koscielny et al., 2018b; Pokharel et al., 2020; Zhu et al., 2021; Kourani et al., 2022). Reproductive organs are highly vulnerable, heat stress results in reduced male and female reproductive viability, asynchronous development, pollen sterility/abortion, failure of pollination/fertilisation and pod abortion (Polowick and Sawhney, 1988; Angadi et al., 2000; Young et al., 2004; Wu et al., 2021; Lohani et al., 2022). It has been estimated that every 1 °C temperature increase from the optimum causes a 10 % yield reduction in OSR (Nuttall et al., 1992). The intensity, timing and duration of heat stress exposure govern the impact on reproduction to high temperature. Even short spells of heat stress at a crucial reproductive stage can be detrimental to seed yield and quality. Various studies have indicated that high-temperature stress can occur at >25 °C in OSR, with >29 °C leading to >50 % reduction in seed yield (Angadi et al., 2000; Elferjani and Soolanayakanahally, 2018; Lohani et al., 2022). Previous studies on heat stress on reproduction employed prolonged and primarily moderate heat stress regimes, highlighting an urgent need for studies focussing on the impact of short episodes of extreme heat stress for OSR productivity (Lohani et al., 2022). There has also been limited studies on high night-time temperature (Pokharel et al., 2020) and minimal studies on cold stress during flowering, with no low chill (<10 °C) day temperatures (Singh et al., 2008; Qin et al., 2023). This study therefore focuses on short episode (3 days) heat stress with high day and night temperatures to mimic short heat wave condition within a temperate location, or cold stress with low chill day and night temperatures. No studies have directly compared both heat and cold (chill) stress at the same time in the same accessions over all these parameters, therefore this study provides a novel approach of looking at temperature stress over a large population base.

With rising demand, crop improvement is vital to boost productivity amid climate change. This study evaluated contrasting *B. napus* (OSR) genotypes to identify cultivars tolerant to short episodes of cold and high temperature stress, during early flower development, a highly sensitive stage. Genetic diversity panels, represent ideal resources for associative transcriptomics (AT) studies to identify markers associated with trait variation, and has been used successfully for traits such as aluminium stress (Du et al., 2022), and freezing stress (Huang et al., 2020). Here we were able to identify regions of interest as markers for breeding for increased yield and improved tolerance to stress, as well as genotypes which may be useful for hybrid production.

2. Results

2.1. Response of Brassica napus to temperature stress

To discover the effect of heat and cold stress on *B. napus* (OSR), a set of 94 accessions were chosen from the BnASSYST panel that represents the diversity of this species (Havlickova et al., 2018). Plants were independently subject to the two temperature regimes, at seedling stage (GS1; heat stress), vegetative growth (GS2; cold (freezing) stress), and during flowering (GS6; heat and cold (chilling) stress), and 8 phenotypic features were collected (Fig. 1; Table 1). Seedling and vegetative analysis were performed to determine if tolerance to heat/cold at earlier stages could be directly correlated to impacts of heat/cold stress at later flowering stages, and therefore enable rapid large-scale phenotyping for breeding traits.

These 8 traits were collected, and extensive phenotypic variability observed among the 94 BnASSYST lines, with heat stress showing a general negative effect. Principal component analysis (PCA) (7 traits; Table 2) was performed to understand the main factors contributing to the phenotypic variability among *B. napus* genotypes. Based on a plot of the top two variables, these explain 59.6 % of the variability for all parameters (Fig. 2A). From the PCA plot it is apparent that while control and cold intermingle, there is a clear separation of the impact of heat. If displayed by type of *B. napus* cultivar, these are also sorted into two separate groups which represent those that require vernalisation (winter, exotics, swede) and those that do not require vernalisation (spring) (Fig. 2B). This suggests that it may be worthwhile comparing the two different groups separately to allow separation of the effect of heat from variety type.

We subsequently investigated the correlation between the different phenotypic variables in the control and stress treatments. We observed an expected strong correlation between seed yield variables, for example positive correlation between seed weight and seed number per pod (SNPP) on the main raceme in both the spring and winter varieties (Figure S1, Table 1) and a strong negative correlation between pod sterility with the other seed variables, e.g. seed yield and SNPP. Seed yield was also negatively correlated with stress, with −0.17 ($P = 9.33e\text{-}09$) in the whole plant and −0.47 ($P = 1.26e\text{-}61$) for the main raceme.

As well as the 8 phenotypic traits, we also recorded date and average day temperature at the start of flowering to see if there was any correlation between timing/temperature and how the lines respond to heat/cold stress. We observed differences between winter/spring, with spring lines having a higher positive correlation between start of flowering date and day temperatures up to flowering, in comparison to the winter varieties. Day temperature also had a strong negative correlation with seed area in the spring varieties, which is not observed in winter varieties, suggesting that time of flowering and seed yield variables are impacted more strongly by environment than the winter varieties, where time after vernalisation may be a stronger variable (Fig. S1A, D).

Differences between treatments (control, heat, cold), were also seen, for example seed yield from the main raceme and total number of pods has a strong positive correlation in winter but not in spring varieties during heat stress (Fig. S1C, F). This suggests that heat may have different effects in the spring and winter varieties. Seed number and area from the main raceme are also weakly positively correlated in heat in the spring, but not in the winter varieties.

2.2. Brassica napus yield is more sensitive to heat stress

Seed yield factors appear closely correlated, average seed yield showed significant cultivar variability with a ~20 g range between min/max (per plant; ~5 biological replicates), with control and cold stress having similar averages (24.8 g and 23.7 g respectively) (Fig. 3A). In heat stress, however, there was a significant decrease (1.3-fold drop; $P = 0.0009$) in seed yield in all genotypes (19.3 g). This was particularly prominent when comparing seed yield from 15 pods from the main raceme with control (0.8 g), cold (0.7 g), and heat (0.2 g; $P < 0.0001$) with a 4.95-fold drop in yield (Fig. 3B). Reproductive development was therefore very sensitive to a short high temperature stress, while cold temperature stress did not have a lasting effect on plant growth and seed yield in our study.

Volcano plots showing significant *P*-values (above dashed line) for whole plant (C) and for 15 pods from the main raceme (D) in heat compared to control. Blue line shows no change, with positive values (right) show increase in seed yield (right), while negative values (left)

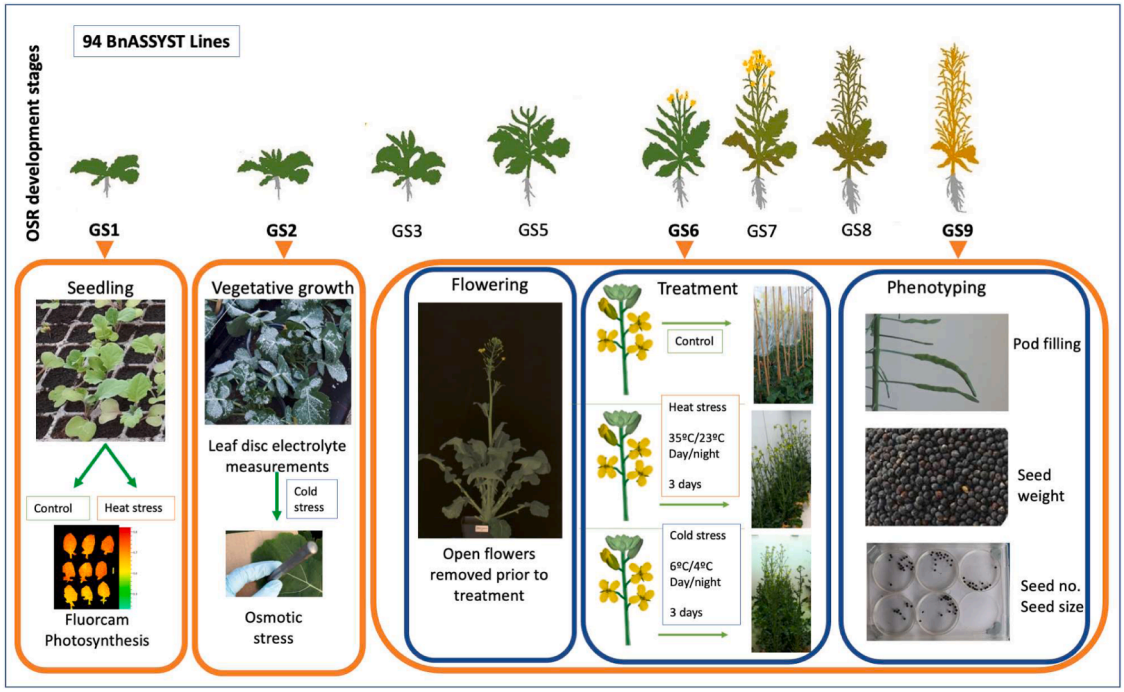

Fig. 1. Infograph showing phenotypic measurements of 94 BnASSYST lines during *B. napus* life cycle. 2 week old seedlings (GS1) were subjected to 3 day control or heat stress (35 °C) and then photosynthesis measurements observed using FluorCam. In the winter genotypes during vegetative growth (GS2), leaf discs were collected and subjected to a freezing stress (−5 °C) to observe electrolyte leakage. Flowering (GS6) plants were subjected to a 3 day control, heat (35 °C) or cold stress (6 °C), then phenotypes were collected at plant senescence (GS9), pod filling, seed weight, number and size. OSR development stages adapted from 'Principle growth stages (BBCH system)'; AHDB (adhb.org.uk/knowledge-library/oilseed-rape-growth-guide).

Table 1
Summary of main correlations for heat and cold stress on the *B. napus* varieties.

	Trait	Pod Sterility		SNPP Main		Seed yield		Total Pods	
Trait	Treatment	Winter	Spring	Winter	Spring	Winter	Spring	Winter	Spring
Seed Yield Main	Control	−0.86	−0.47	0.92	0.82	0.37	0.37	−0.26	−0.05
	Cold	−0.47	−0.32	0.82	0.94	0.37	0.63	−0.05	−0.09
	Heat	−0.86	−0.60	0.92	0.72	0.37	−0.10	0.53	0.11
SNPP Main	Control	−0.72	−0.63	–	–	0.44	0.23	0.08	−0.05
	Cold	−0.63	−0.51	–	–	0.23	0.53	−0.05	−0.19
	Heat	−0.72	−0.50	–	–	0.44	0.29	0.08	0.20

show a decrease in seed yield compared to control.

Comparison of seed yield in whole plant (x) to 15 pods from the main raceme (y) (E), showing significant different trend in heat (0.31), in comparison to control (0.53) and cold (0.51).

When comparing seed yield for individual genotypes in the whole plant to 15 pods from the main raceme, we can see that control and cold have a clear correlation with very similar R^2 scores (0.53 and 0.51 respectively) (Fig. 3E). Using a Pearson's correlation test, we have shown that there is a high correlation between these two seed yields in control 0.63 ($P = 3.18e-43$). This suggests that main raceme seed yield is a good proxy for final whole plant seed yield, indicating that plants that yield better on their main raceme, also have better yield on the rest of the racemes. This trend was different in the genotypes subjected to heat stress, they had a lower correlation, suggesting other factors may be affecting seed yield in the whole plant; Pearson's correlation = 0.48 ($P = 3.37e-61$). For example, more visible impact on the developing flowers on the main raceme, but not on the secondaries where sensitive developing flowers may not be present during the heat stress. Additional branching to compensate for the loss of the main raceme yield, may also affect whole plant yield. Two genotypes (BnA510, 105; Winter OSR) appear as outliers with high yield in main raceme and whole plant under heat stress, and therefore show increased tolerance (Fig. 3E).

Seed yield in the different genotypes cluster into high and low yielding cultivars under control conditions (Fig. S2A,B). Within these clusters we have genotypes that are tolerant or sensitive to the stress conditions, in cold stress the majority show no statistical difference with <10 genotypes showing an increased/decreased seed yield (Fig. S2C-D). Similarly in whole plant seed yield most heat stress genotypes are not significantly changed (tolerant), 3 genotypes have increased yield (resistant), while >20 genotypes have decreased yield (sensitive) to heat stress (Fig. 3C). However, when just observing the main raceme, only 5 genotypes are not significantly changed (tolerant) compared to control, with all the others showing significant decreased yield under heat stress (Fig. 3D). From the heat map (Fig. S2B) there are two small clusters showing some tolerance (8 genotypes), and slight tolerance (12 genotypes) to heat stress. The heat map also shows a clear cluster of four genotypes that are sensitive to both cold and heat stress, 3 of which are spring genotypes (Fig. S2B). Suggesting that spring genotypes may be more sensitive to stress than winter genotypes.

2.3. *Seed number and pod sterility appear to have the strongest influence on seed yield in heat stress*

The percentage of filled pods per raceme, seed size and seed number

Table 2
Phenotype information.

Phenotype ID	Phenotype	Information
No_Pods	Number of pods	Plants were subjected to control, heat (35 °C) or cold (6 °C) stress during flowering (GS6). At senescence (GS9) the number of pods were counted on the main raceme
Pod_Sterility	Percentage of pod sterility	Plants were subjected to control, heat (35 °C) or cold (6 °C) stress during flowering (GS6). At senescence the percentage of pods that were sterile (unfilled) on the main raceme were recorded
Elec_Leakage	Electrolyte leakage	At GS2, winter OSR lines were subjected to cold stress (<4 °C). Leaf disc's were collected and subjected to freezing conditions (−5 °C) and amount of electrolyte leakage was measured
QY_Max	Photosynthesis	2 week old seedlings (GS1) were grown in growth room (21 °C) or heat (35 °C) stress for 3 days and FluorCam used for photosynthesis measurements
Plant_Yield	Whole plant seed yield	Plants were subjected to control, heat (35 °C) or cold (6 °C) stress during flowering (GS6). At senescence yield (seed weight (g)) from whole plant was recorded
Main_Yield	Main raceme seed yield	Plants were subjected to control, heat (35 °C) or cold (6 °C) stress during flowering (GS6). At senescence yield (seed weight (mg)) from 15 pods collected from main raceme
Seed_No	Seed number	Plants were subjected to control, heat (35 °C) or cold (6 °C) stress during flowering (GS6). At senescence average number of seeds from 15 pods collected from main raceme
Seed_Size	Seed size	Plants were subjected to control, heat (35 °C) or cold (6 °C) stress during flowering (GS6). At senescence average size of seeds from 15 pods collected from main raceme

can all influence overall seed yield (weight) and these factors correlated strongly together (Fig. S1, Table 1). The different factors were therefore analysed in detail using distribution histograms of frequency values (Fig. 4). The histogram plot highlights the variation within the genotypes, cold stress having a similar range compared to control conditions, with a minor negative effect on phenotypic variables, heat stress however has a significant negative effect (Fig. 4). Here we can see a clear drop in seed weight, seed number per pod, and an increase in pod sterility in the main raceme under heat stress.

Volcano plots showing significant *P*-values (above dashed line) for main raceme showing seed number (E) and percentage of sterile pods (F) in heat compared to control. Blue line shows no change, with positive values (right) show increase (right), while negative values (left) show a decrease compared to control.

However, looking at individual genotypes, there is less variation in average seed size, with clustering of those genotypes with smaller seed size under heat stress, 14 which are significantly decreased (Fig. S3A, D). In cold, all except 2 lines showed non-significant changes, suggesting seed size does not have a major effect on overall plant yield. This is also observed in the correlation matrix, with seed size having little correlation for the winter genotypes (Fig. S1). In the spring genotypes, seed size is strongly correlated with flowering time and temperature, suggesting there is more variability within the spring genotypes. Seed size is also correlated with pod sterility in the heat only, suggesting the amount of sterility in pods may be linked to the smaller seed size observed in heat stress.

Seed number however appears more sensitive to stress, with a cluster of 10 genotypes with reduced seed number/pod in both cold and heat stress (6 spring), suggesting these genotypes may be generally sensitive to abiotic stress (Fig. S3B). In heat stress, most genotypes have reduced seed number (sensitive), 11 non-significantly changed (tolerant), and 1 genotype had increased seed number/pod (resistant) (Fig. 4E).

Pod filling showed an increased percentage of sterile pods in heat stress for all genotypes (Fig. 4D), with only a few able to maintain pod filling under heat stress (8 genotypes) (Fig. 4F, S4B). Similar, to other analysis, there is a small cluster (5 genotypes) which have increased percentage sterility in both cold and heat stress, while most lines are tolerant to the cold (Fig. S4B, E).

Cultivar and the stress treatment caused significant changes in pod number in the main raceme in comparison to control, with heat stress inducing more extreme changes (Fig. S4A-C). In heat stress there were two alternative responses, producing two separate clusters, with some having reduced pod number (these were highly sensitive to heat treatment and tended to result in the death of the main raceme – 12 genotypes), or those that had an increased number in pods produced (16 genotypes), possibly as a way to combat the stress and maintain seed production (Fig. S4A,C). In comparison cold stress only had a limited effect on pod number, with the majority not being significantly affected (Figure S4C).

2.4. *Stress markers identify lines that are tolerant to heat and cold stress*

As stress, especially heat stress, effects reproduction, the ability to have an early stress marker as a phenotyping tool would be beneficial, therefore we looked at the correlation between early markers and final yield data. Focusing on heat stress in seedlings (GS1), and freezing stress during vegetative growth (GS2).

Chlorophyll fluorescence has been demonstrated as a high-throughput phenotyping screen in rice for heat tolerance (Robson et al., 2023). In this study we have shown that photochemical efficiency of PSII (Fv/Fm) was positively affected by heat stress (Figure S5B) at the

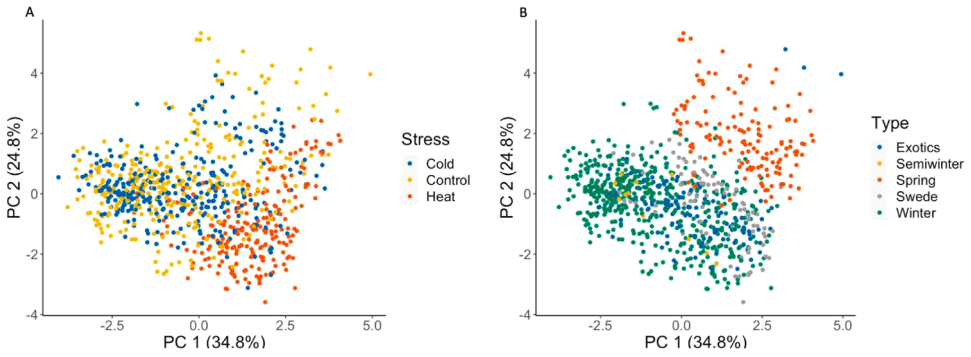

Fig. 2. Principal component analysis (PCA) biplot for the first two principal component (PC) scores, PC 1 vs PC 2 for 7 phenotype traits (No_Pods, Pod_Sterility, QY_Max, Plant_Yield, Main_Yield, Seed_No, Seed_Size, see Table 2 for trait description). A) showing plot coloured by type of stress treatment, and B) showing plot coloured by *B. napus* variety type.

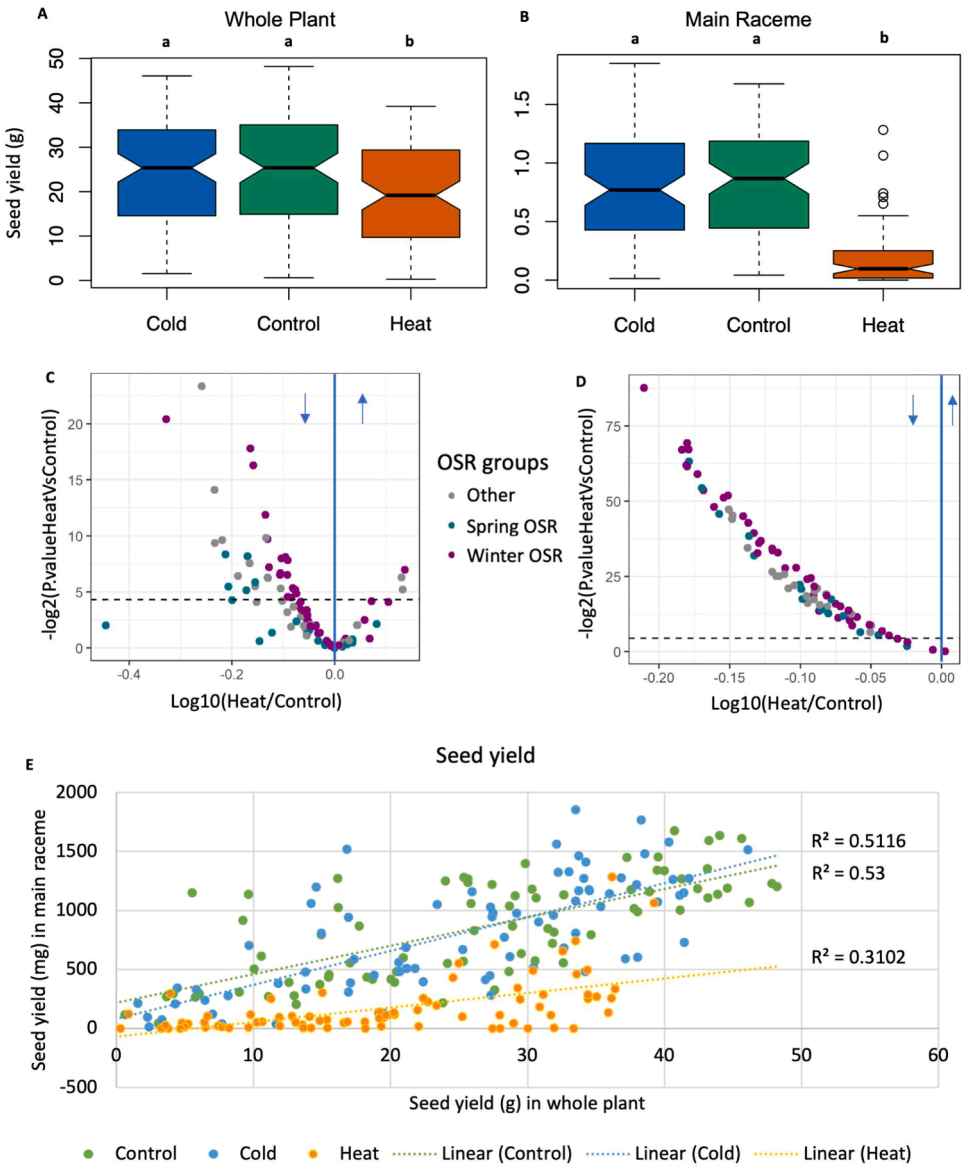

Fig. 3. Average seed yield in control, cold and heat treatments. Seed yield (g), for the whole plant (A), and for 15 pods from the main raceme (B). Median is marked by the dark line, interquartile range is marked by the box, whiskers show maximum and minimum scores with outliers represented by circles. Values followed by the same letter are not significantly different from each other (Student T-Test < 0.05).

seedling stage, with a significant increase in QYmax after heat stress in all genotypes except 4 (Figure S5C). Therefore, these conditions were not significantly stressful at the seedling stage to damage the photosystem. The correlation plots show that the cultivar's responses to heat stress in seedling photosynthesis do not correlate to yield characteristics (Figure S1). Interestingly 3 of the genotypes with highest photosynthesis under stress clustered with heat tolerant varieties (Fig. 5), and also had increased resistance to the freezing stress (e.g. BnA213 an exotics winter oilseed rape line), suggesting that there may be mechanisms in place that provide general tolerance for both stresses and in all developmental stages.

Studies have shown that under low temperature the permeability of cell membranes and electrolyte leakage can increase, resulting in increased relative conductivity of tissues, therefore electrical conductivity can be used as an index to determine the degree of frost damage (Huang et al., 2018). In the heatmap (Fig S5A), most genotypes show ~0 which suggests these genotypes had a high level of damage during the freezing stress, a small cluster (11) show a tolerance to freezing with a smaller loss in electrolyte leakage under freezing stress. No correlation was observed in the whole data set, therefore unfortunately for the majority the effect of stress in seedling and vegetative growth does not appear to be representative of how *B. napus* will respond during reproduction and therefore cannot be used as an early stress markers.

2.5. *Individual's genotypes response to heat stress*

From this data it is obvious that while cold has a limited effect, except on a few genotypes, all genotypes observed are affected by heat stress to different degrees. To analyse which genotypes may be more tolerant to heat stress, we used heatmap and clustering based on trait differences in heat compared to control (Fig. 5). Clustering of two groups was evident, the larger group cluster C and D where there are a lot of genotypes below average (blue), indicating more negatively affected by the heat and suggesting heat sensitive genotypes. A smaller group of clusters (A and B) which overall have a higher positive value compared to average (red), these are the genotypes that are less affected by the heat and suggest heat tolerance to varying degrees (28 genotypes) (Fig. 5). A few of these genotypes BnA105, 518, 98, 106, and 510 were

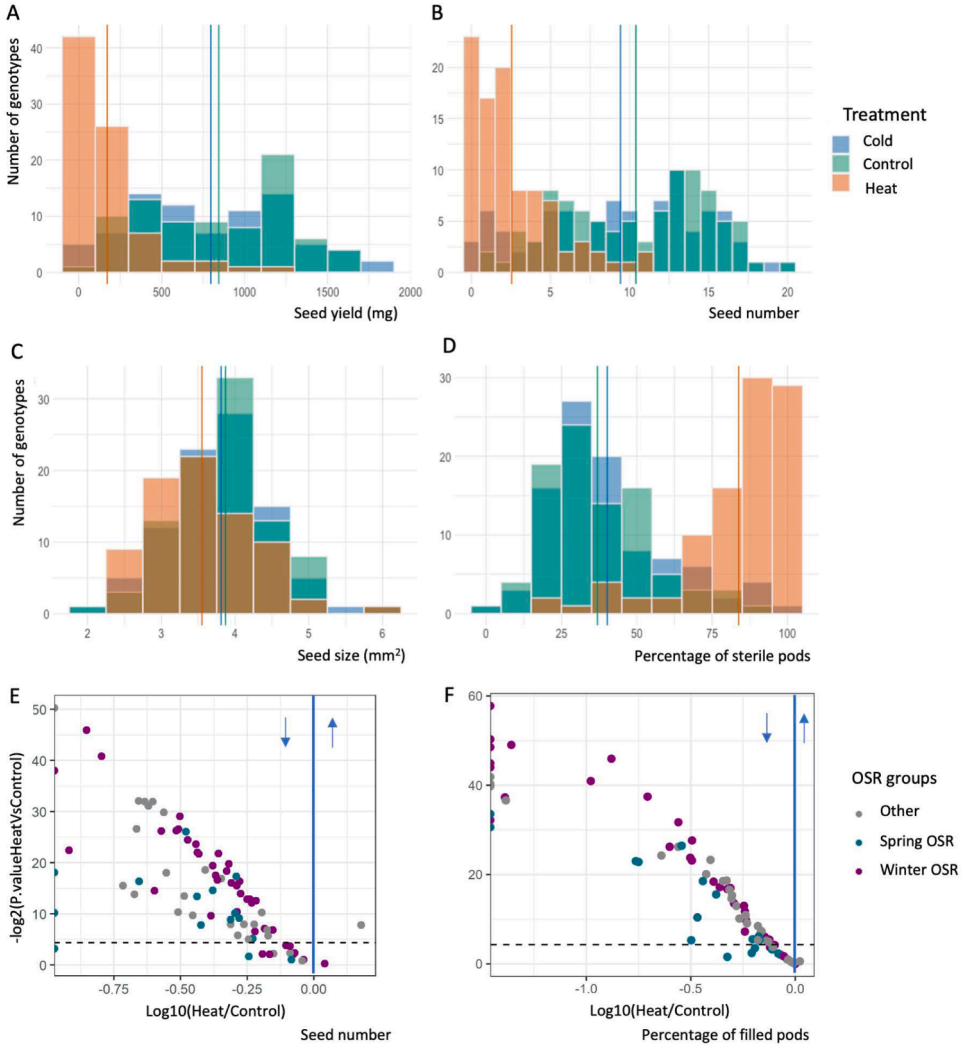

Fig. 4. Distribution histogram of frequency of different values in the main raceme, in the control, cold and heat treatment, for seed yield (A), seed number (B), seed size (C) and pod sterility (D). Single lines show mean value for heat (orange), cold (blue) and control (green).

already identified as genotypes that were able to have a high seed yield despite the heat stress conditions (Fig. 3E), and are all winter OSR, with the majority in cluster A.

2.6. *Association Transcriptomics identified loci associated with yield and yield recovery*

To investigate the genetic bases of these traits and responses to environmental stress, we employed a genome wide-association study (GWAS) approach. An Associative Transcriptomics (AT) analysis pipeline combining GWAS and Gene Expression Marker (GEM) analysis (GAGA; (Nichols, 2022)) was used to identify single nucleotide polymorphism (SNP) and GEMs associated with yield and resilience traits. We performed AT analysis using phenotypic traits, seed number in the main raceme and seed weight on the whole plant. We first looked in the control conditions to understand more about individual genotypes of *B. napus* and the correlation between seed weight and seed number (Fig. 6A-C). This suggests that winter varieties of *B. napus* have the highest seed weight and seed number, in comparison to the spring and exotic/kale/other genotypes, with seed number and seed weight on the main raceme having a high correlation of $R = 0.706$ in control conditions. The combined effect of these two traits underlies "seed yield". Thus, we performed a meta-analysis (METAL analysis; (Willer et al., 2010)) which combines evidence for association from both traits using appropriate weighting to identify markers associated with seed yield. Manhattan plots of seed yield showed significant marker-trait association from GWA and GEM analysis on Chromosome 4 on the A and C genome. Linkage decay indicates 0.2mb region covering 37 genes ($R^2 > 0.2$) around the main marker, Cab035024.1.1.1065.C on A04, and 0.39mb region covering 56 genes ($R^2 > 0.2$) around the main marker, Bo4g186210.1.6486.C, on C04 (Fig. 6 D-E, H-I). Segregation of seed number (Fig. 6F) and seed weight (Fig. 6G) with the highest associating markers: Cab035024.1.1065.C (A04) and Bo4g186210.1.6486.C (C04), shows a significant difference. This suggests that these markers may be involved in seed set with a negative correlation. For a full list of all potential underlying genes see Supplemental Table S3.

Further analysis was conducted on the response of *B. napus* to heat stress, by looking at seed recovery by comparing ratios between control and heat stress in both the main raceme and in the whole plant. As established earlier, the main raceme was more negatively affected than the whole plant (Fig. 7A), however unlike previously (Fig. 6A-B), we see that there is a broader spread of crop types, with spring genotypes having a noticeable shift to higher differences in ratio suggesting that these may be able to recover from the heat stress better (Fig. 7A). Manhattan plots for recovery of seed yield showed significant marker-trait association from GWA analysis on Chromosome 1 on the C genome. Linkage decay indicates 1.02mb region covering 206 genes (R2 > 0.2) around the main marker Bo1g002670.1.2184.G on C01 (Fig. 7D-

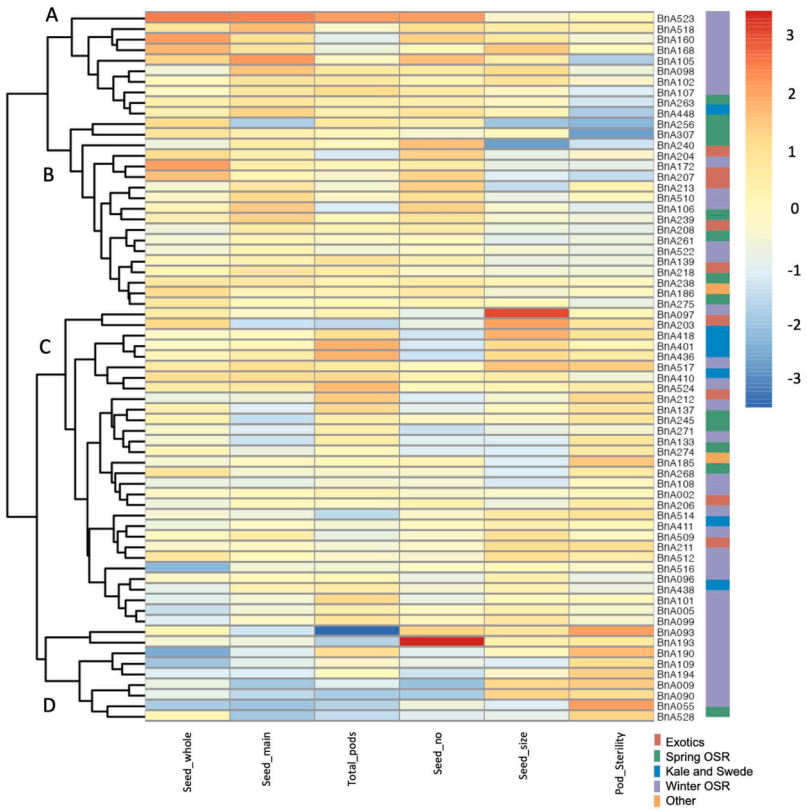

Fig. 5. Normalised heatmap based on the average difference between Control and Heat for each phenotypic factor. Blue showing below average (higher negative difference) and red showing higher positive difference.

E, H-I). Segregation of the recovery of seed yield after heat stress with the highest associating markers: Cab020546.2.7671.T (A01) and Bo1g002670.1.2184.G (C01), suggests that these genes may be involved in seed recovery from heat stress; for a full list of all potential genes see Supplemental Table S4. Included within this list are 50 genes which are responding to stimuli, including Hsp40/DnaJ family protein which is known to be a heat shock chaperone protein, and therefore interesting targets for improving heat tolerance.

3. Discussion

Extreme weather events are having major impacts on global crop production; losses of 1.19 billion metric tons of cereals have been recorded due to extreme heat between 1964 and 2007 (Lesk et al., 2016), economic losses within the EU of nearly €650 billion since 1980–2022 due to extreme weather with 20 % due to heat waves (EEA., 2022; https://www.eea.europa.eu/en/analysis/indicators/economic-losses-from-climate-related). These increasingly unpredictable and extreme weather events mean that *Brassica napus* (OSR) can be subjected to both cold and heat stress throughout its growing cycle and during its sensitive reproductive development. In the UK, global temperatures have been steadily increasing, with the last 20 years having the 10 warmest years, with a trend of an increase in 1 °C on average and an increased frequency of extreme temperatures events. Extreme weather events can last a few days, with >5 °C above or below-average temperatures (Lohani et al., 2022). In July 2020 a highest maximum temperature of 37.8 °C was observed within the UK, which was >20 °C above the UK mean temperature for that month, while in March 2021 the lowest minimum temperature of −8.5 °C was recorded, which was >14 °C below the UK equivalent monthly mean temperature (metoffice.gov.uk/research/climate/maps-and-data/summaries/index, accessed 2023). In response to temperature stress, plants can undergo three main coping strategies: avoidance, escape and tolerance. We performed a large-scale phenotypic experiment to identify tolerance in *B. napus* to cold and heat stress. Breeding tolerance into crops is a crucial agronomic trait to prevent yield losses. High temperature stress causes rapid physiological, biochemical and molecular responses which confer tolerance to some extent when crop plants are gradually or briefly exposed to moderate or high temperature, but if the intensity, frequency or duration of exposure is too high, the effects are detrimental (Zhu et al., 2021). While cold temperature stress generally leads to development slowing down, negatively influencing photosynthesis and respiratory metabolism, leading to lower yields (Thakur et al., 2010).

Previous research has shown that seed yield is highly sensitive to temperature stress, especially during the reproductive stage; most studies have looked at long-term heat stress (>7 days), with only a few short-term heat stress studies. We choose a short-term heat stress (<4 days) as being more typical of an extreme temperature event in the UK (heat wave/cold snap). Recent work by (Pokharel et al., 2020) suggested that reproduction is also sensitive to high night-time temperatures (>20 °C), therefore we kept a high night-time temperature in our heat stress, which is also a common occurrence during these extreme temperature events. Through this study, we have shown that while the majority of OSR genotypes were tolerant to cold (Fig. 3), a few lines were sensitive to cold with reduced values in main raceme seed weight and seed number, the majority of these were spring varieties (e.g. BnA256, 261, 270), which do not grow overwinter, but also included a swede (BnA448) and two winter varieties (BnA160, 516), which may be more sensitive to environmental fluxes. Previous research has shown a direct negative effect of cold stress 12/2 °C (day/night) on pollen viability, stigma receptivity and pollination (Qin et al., 2023). However, in our case the majority while may have had short-term effect of the cold stress in these developmental processes, there was not a significant effect once the stress was over. The cold stress appeared to halt flower development during the stress, with less flowers per day being produced, but they returned to normal once control conditions were reestablished and

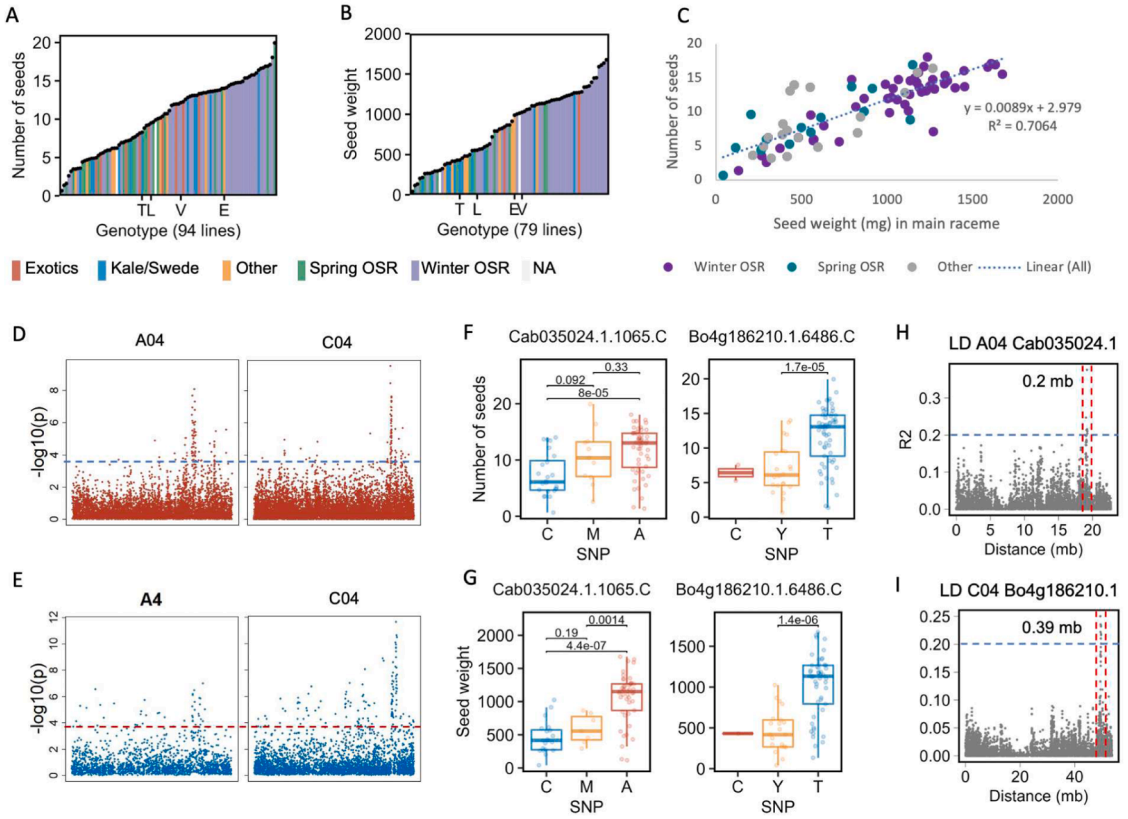

Fig. 6. Seed yield (number and weight) in *Brassica napus* genotypes under control conditions. Seed number (A) and seed weight (B) on main raceme in 94 *B napus* genotypes (bar graph, colours indicate crop types), genotypes (BnA194; E), (BnA102; L), (BnA436; T) and (BnA053; V) shown on both graphs. (C) Linear regression analyses of seed number and seed weight in the *B. napus* genotypes. Manhattan plots showing chromosomes with the most significant marker-trait association resulting from GWA (D) and GEM (E) analyses of seed yield (METAL analysis combining seed number and seed weight). The x-axis indicates GEM or SNP location along the chromosome; the y-axis indicates the -log10(p) (*P* value), dashed lines indicate FDR > 0.05 cut-off values. Segregation of seed number (F) and seed weight (G) with the highest associating markers: Cab035024.1.1065.C (A04) and Bo4g186210.1.6486.C (C04). *P* values were determined by a Student's *t*-test. Linkage decay of plot markers Cab035024.1.1065.C (A04) (H) and Bo4g186210.1.6486.C (C04) (I) as a function of genetic distance (mb). The blue line indicates an R^2 value of 0.2, red lines indicate the area of linkage disequilibrium.

exhibited good seed set. It is possible that the duration of the cold stress, or the severity of the stress, needs to be increased to determine those lines that show moderate or high tolerance to cold stress during reproduction. In comparison all lines had some degree of sensitivity to heat stress, with a significant drop in yield during the heat stress as well as after the heat stress in the majority of lines.

Seed yield is the total of the number of seeds per plant and their weight, and this is influenced by the number of pods per plant, percentage of filled pods, number of seeds per pod and seed size. Associative Transcriptomics (AT) analysis can identify important markers involved in seed yield for line improvement. This AT pipeline has been successfully applied to diverse phenotypes including flowering time and resistance to multiple pathogens (Harper et al., 2012; Jacott et al., 2024). Here we have identified a significant marker-trait association from GWA and GEM analysis on Chromosome 4 on the A and C genome, linked to seed yield. Yield is a highly plastic trait subject to large environmental and genotype by environment effects. Therefore, the discovery of QTL associated with yield will be highly influenced by study environment and the genetic panel used for trait association. Due to the importance of seed yield traits a number of studies have identified >100 QTLS, with major QTLs located on A1, A6, A7, A9, C1 and C9 (Yang et al., 2012; Shi et al., 2015; Miller et al., 2019; Zhu et al., 2020; Pal et al., 2021; Xiang et al., 2023; Zhang et al., 2023). While our marker does not correspond to any known markers associated with yield, some of the genes identified are linked to yield related studies (32/56 – GWAS or QTL identified (Dong et al., 2023); Supplemental Table S3) and four of the candidate genes are known to be involved in yield related traits in Arabidopsis or *B. napus* (GAMMA-TIP1, RAX2, ATPGP1 and SAM1 (Raboanatahiry et al., 2018; Dong et al., 2023); Supplemental Table S3). RAX2 and SAM1 are involved in organ development, leaf and flower respectively, therefore both of these genes have a direct effect on yield through number of leaves and therefore energy availability (photosynthesis potential), and number of flowers and therefore number of pods (Müller et al., 2006; Hu et al., 2023).Thus suggesting a possible new important marker for breeding when looking at combined seed traits. Transcriptomics for gene expression marker analysis was conducted on the second true leaves and therefore represents the standard background level of gene expression during vegetative growth. It does not reflect the reproductive stage or conditions of environmental stress. Despite this difference in developmental stage and treatment, it has been successfully used to identify genes involved the control of traits in other tissues and environments such as seed fatty acid composition (Havlickova et al., 2018) or oil content (Miller et al., 2019). ShinyGO analysis of this region identified several enriched pathways, such as zeatin biosynthesis which is directly related to seed yield, influencing seed number and size through cytokinins (Jameson and Song, 2016) (Supplemental Table S3). Further work looking at the environmental effects on gene expression on lines of interest would further help elucidate genetic responses to heat and cold stresses that may impact on yield.

High temperature stress during flowering influenced several of the seed yield components, with less overall seed yield (whole plant and main raceme), reduced number of filled pods, reduced number of seeds per pod, and decreased seed size (Fig. 3–4). Our data agrees with other heat stress experiments, with a marked reduction of seed production

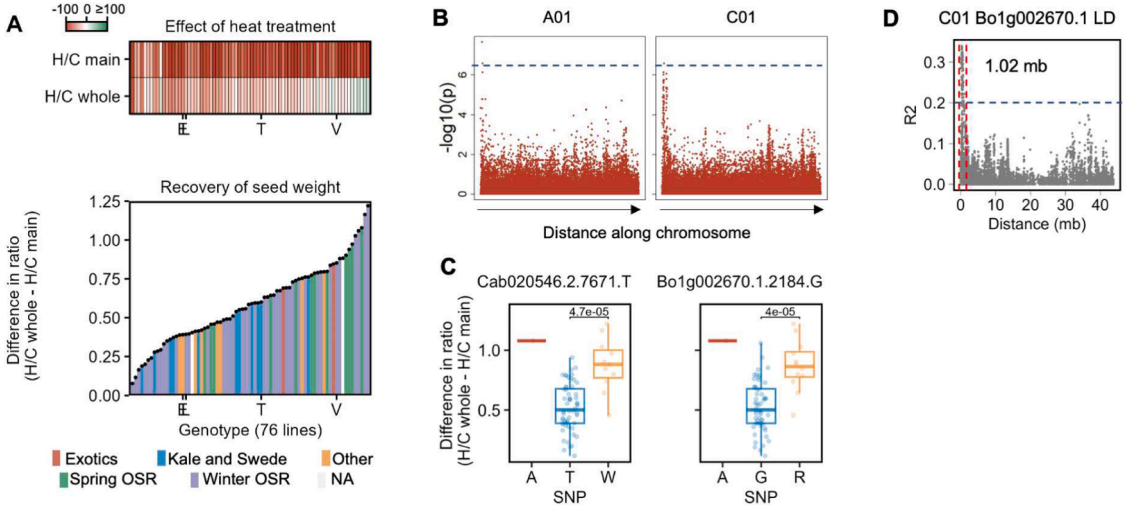

Fig. 7. Recovery of seed weight after heat treatment in *Brassica napus* genotypes. (A) Heatmap showing the effect of heat stress treatments (compared to control treatment) on seed weight from the main stem and whole plant for 76 *B napus* genotypes. Bar graph below indicates the recovery of seed yield after heat stress as the difference between the heat-effect ratios (heat/control treatment, H/C) for the whole plant and main raceme. Crop types are indicated in colours, genotypes (BnA194; E), (BnA102; L), (BnA436; T) and (BnA053; V) shown on both graphs. (B) Manhattan plots showing chromosomes with the most significant marker-trait association resulting from GWA analysis of the recovery of seed yield after heat stress. The x-axis indicates SNP location along the chromosome; the y-axis indicates the -log10(p) (*P* value); the dashed line indicates FDR < 0.05 cut-off value. (C) Segregation of the recovery of seed yield after heat stress with the highest associating markers: Cab020546.2.7671.T (A01) and Bo1g002670.1.2184.G (C01). *P* values were determined by a Student's *t*-test. (D) Linkage decay of plot marker Bo1g002670.1.2184.G (C01) as a function of genetic distance (mb). The blue line indicates an R^2 value of 0.2, red lines indicate the area of linkage disequilibrium. There was no LD with marker Cab020546.2.7671.T (A01).

after short-term heat stress (53–68 % loss) (Lohani et al., 2022), and longer-term heat stress (81–96 % loss) (Angadi et al., 2000), with decreased seed number per pod (SNPP) (Angadi et al., 2000), and seed size (Lohani et al., 2022). From the seed yield data, it is obvious that for the majority the whole plant coped with the heat stress better than the main raceme. And this may suggest that the plant can use different mechanisms to survive short-term heat stress. For example, plants can prolong the growth of the main raceme, develop more branches and increase their flowering time to compensate for increased seed abortion (Angadi et al., 2000; Lohani et al., 2022; Mácová et al., 2022). We also observed this with a significant increase in pod number after heat stress (Fig. 5). We also noted that there was immediate increased flower development speed in heat stress, with up to 15 flowers/day opening in comparison to 4–5/day in control and 1/day in cold stress. Research has shown that generally, all developmental decisions and phase transitions are accelerated under high temperature (reviewed by Seth and Sebastian (2024)). This suggests that temperature may have a direct effect on pollen and/or embryo development, e.g. early acceleration (Lohani et al., 2022), which may cause a further mis-regulation of essential signalling pathways leading to pollen/embryo sterility. In our study and previous research (Angadi et al., 2000) the flowers that opened during the heat stress did not produce any fertile pods, showing that pollen/-ovule/embryo development, pollen germination or fertilisation is highly sensitive to heat stress. In the most sensitive genotypes, we observed main raceme growth arrest, loss of apical dominance was compensated by an increase in secondary racemes. Angadi et al. (2000) also noted that while more floral primordia developed due to heat stress, those developing during or after heat stress may not develop into normal flowers or pods. It is therefore evident that heat stress has a negative prolonged effect on the plant after heat stress. This reduction of seed yield could reflect several different physiological changes to the plant such as reduced flower fertility (pollen/ovules), and fertilisation success. Research has shown that pollen development (microsporogenesis) could still occur under HS, but viability, germination & pollen tube growth was reduced, female gametophyte development is also affected by heat stress (Young et al., 2004; Rahaman et al., 2018; Kourani et al., 2022). Reciprocal crosses, between heat stressed and control showed that for Brassica both female and male are important for the negative effect of heat stress, with a reduction of seed set by 88 % for HS pollen and 37 % for HS ovules (Young et al., 2004). There was also no seed produced from fertilisation events up to 4 days prior to the onset of heat stress, suggesting post-fertilisation events (e.g. megagametophyte development) were also negatively affected (Young et al., 2004). These developmental processes are a highly energy demanding process and therefore reduced photo assimilate supply will affect these stages as well as having a reduced capacity of the plant to support pod/seed development (Ferguson et al., 2021). Photosynthesis has been shown to be affected, with a reduction in PSII photochemical efficiency (Fv/Fm) and an increase in thylakoid membrane damage (Fo/Fm) under heat stress (Pokharel et al., 2020). Investigating these features in the future would be valuable to discover the most sensitive factors of the prolonged effect of the short-term heat stress, as possible mechanisms of mediating/creating more tolerant plants.

From our study there is a high variability in the number of filled pods within the different genotypes even in control conditions, with an average filled pods of 73 % on the main raceme. Morrison and Stewart (2002) have previously shown that OSR plants produce nearly twice as many flowers as they fill (56 % for *B. napus*), which they suggested is a survival mechanism to cope with loss. This has been seen in other research with an increase in empty or shrivelled pods (Morrison and Stewart, 2002; Lohani et al., 2022), leading to higher pod number as observed in our study.

Selecting tolerant genotypes is considered the best strategy to reduce negative stress effects (Secchi et al., 2023). Through this screen we have shown that 28 genotypes that show an increased trend for more tolerance to heat stress, with 5 of these genotypes (Winter OSR; BnA098, 510. Winter hybrid OSR; BnA105, 106, 518) identified as genotypes that were able to have high seed yield despite the heat stress conditions. These 5 genotypes seem to have a similar mechanism for tolerance, where they can maintain seed set and pod filling on the main raceme, and retain their yield on the whole plant under heat stress. There does not appear to be a large increase in pod numbers on the main raceme, suggesting an overall tolerance of these genotypes rather than an escape mechanism of producing more raceme and pods once the stress is finished.

Knowing how genotypes respond to heat is not only important for using the correct variety for the environment, but it is also important for breeding strategies for heat tolerance. As demonstrated in this study hybrid lines often have an increased tolerance to abiotic and biotic stresses as well as generally increased yield. Koscielny et al. (2018a) demonstrated that tolerant inbred lines exhibit more tolerance as hybrids, while two susceptible inbred lines created susceptible hybrids, suggesting an additive genetic effect. Therefore, performing screens like the one reported here can inform breeders how to increase phenotypic variation and provide predictive information for hybrid combinations regarding heat stress tolerance, with BnA098 and BnA510 being desirable for heat tolerance in hybrid combinations, thereby providing a valuable tool to accelerate genetic gains within changing environments. In addition, AT analysis can identify markers involved in heat tolerance/seed yield recovery. Heat stress tolerance in plants is complex involving numerous biochemical and metabolic activities, with increased gene expression/translation, protein stability and accumulation of compatible solutes and antioxidants (Rahaman et al., 2018). Therefore, heat stress tolerance can be a polygenic trait making it difficult to identify favourable alleles for breeding. In this study 94 accessions of *B. napus* were used, and when looking at seed yield recovery after stress, identified a region on Chromosome 1 (1.02mb) covering 206 genes, some of which are linked to hormonal and stress response pathways. The DNAJ(HSP40) heat shock domain, HSP40, and mitochondrial heat shock protein 70–1, could potentially play a role in tolerance as HSP play a major role in coordinating transcriptional modifications to heat stress (Seth and Sebastian, 2024), and the pollen development processes is highly energy dependent provided by an increased number of mitochondria during this stage in the tapetum. Hormone pathways are involved in regulating the plants development, homeostasis and stress responses, with SMALL AUXIN UP-REGULATED RNAs (SAURs) involved in the regulation of abiotic stress adaptative growth (Franklin et al., 2011). While the region identified does not align with other previously identified regions or markers (Rahaman et al., 2018; Sandhu et al., 2019) this may reflect trait polygenicity and the differences between spring/winter genotypes, or genetic difference between *B. juneca* (used in previous studies) and *B. napus* tolerance to heat stress. ShinyGO analysis of this region has identified several enriched pathways, with the highest being cyanoamino acid metabolism which plays a crucial role in plant stress response and has been identified previously involved in heat stress response (Liu and Lin, 2020). In this gene list there are also 11 genes involved in yield related traits from either *A. thaliana* or *B. napus* (Raboanatahiry et al., 2018; Dong et al., 2023), suggesting the interplay between yield and stress responses may be important for yield recovery (Supplemental Table S4). Given that the expression level dataset which does not reflect the reproductive stage or environmental stress that are being assessed, flower or stress specific genes may be used to help identification by GWAS and GEM analysis. Expression level datasets created for reproductive development and stress response within accessions of interest may further help elucidate genes and mechanisms involved in stress resilience. Future analysis of the regions identified may identify potential targets for improving heat tolerance both at reproductive and vegetative growth.

3.1. Conclusion

Heat stress had a more pronounced effect on flowering than cold stress, negatively effecting seed yield, number and size, as well as pod filling. We have identified 28 heat-tolerant and 5 high-yield tolerant varieties, which could be used for breeding or growing strategies within the UK to provide for climate-resilience. Two regions involved in seed yield in control conditions, and seed yield recovery after heat stress, have been identified, these regions need to be explored in more detail to identify potential markers for breeding. Future direction into the molecular mechanisms of the heat stress effect on the different processes (e.g. pollen/gametophyte/megagametophyte development) and underlying photosynthesis and photo assimilate availability would be important to discover those most sensitive areas.

4. Methods

4.1. Plant materials

94 genotypes of *Brassica napus* (oilseed rape; OSR) were used in this study (Supplemental Table 1). These genotypes came from the BnASSYST diversity panel and were selected to represent variable geographic origins and provide a wide representation of different OSR types and genomes (Havlickova et al., 2018). Five replicate plants were used for each genotype and treatment to allow for feasible assays with ample replication across each phenotype measured. Nevertheless, for certain phenotypic traits certain lines yielded unreliable data leading to variable numbers of genotypes used for different analyses.

Seeds were germinated in Levington Seed & Modular + Sand – F2S in propagation trays, 1 seed per well. At 3 weeks old, single plants were transferred to 5 L pots containing Levington C2 compost and placed into single skinned polytunnel with no supplementary lighting or heating in September 2018. Pots were watered and feed by automatic irrigation, and plants flowered between Jan-July 2019. After stress treatment, individual plants were bagged to avoid cross-pollination, and the plants within the bags were shaken regularly to ensure self-pollination. The control, heat-stressed and cold-stressed plants were allowed to complete their development within the single skinned polytunnel until seed filling and maturity.

4.2. Cold/Heat stress

At the onset of flowering GS60 (BBCH staging; after the first 5 flowers had opened) plants were transferred to a cold (6 °C/4 °C) or heat (35 °C/23 °C) growth room for 4 days before being moved back to the polytunnel and bagged (Fig. S6). Light cycle was set to 16/8 h (day/night), with humidity 80–100 % in heat and 65–85 % in cold. Lighting system was set up with 8 metal halides 400 W bulbs (Venture Lighting) for cold growth room, and Valoya Bx 180 NS1 190–204 W LED bar (Greenlux Lighting Solutions) for the heat growth room. In both growth rooms temperature control units Heat-Cool were supplied by GAH (Refrigeration) UK) The plants were watered daily or as required to eliminate drought stress as a potential limiting factor.

4.3. Phenotypic characteristics collected

For each treatment (Control, Heat, Cold) five plants from each genotype had measurements collected. Observations were made to examine the effect of temperature stress on the genotypes. Number of days to first flower was measured from sowing date to first open flower. Temperature data was collected using a datalogger and temperature during sampling and flowering period was monitored in both the polytunnel and stress condition growth rooms. After complete seed filling, a number of data points were collected prior to the individual plants being threshed, such as pod number and seed number/size images, and seed weight. The percentage of pod sterility was calculated from the total number of reproductive pods on the main raceme, and the number of sterile pods on the main raceme, providing an indication of the effect of the stress on fertilisation, as pods without seeds tend to abort. The main raceme yield (Main_Yield) was calculated by collecting 15 pods from the main raceme to represent the age range of the pods (5 oldest/bottom, 5 middle, 5 youngest/top), this data plus the yield for the whole plant after threshing was used to calculate the yield for the whole plant (Plant_Yield) (Table 2).

4.4. Electrolyte leakage analysis

An electrical leakage (EC) analysis was carried out by using a

modified version of the procedure described by (Thalhammer et al., 2020). Only the lines requiring vernalisation were growing in freezing temperatures required for this study, so 71 winter genotypes were analysed. Samples were collected during winter with an air temperature <4 °C, and leaf discs (1.4 cm) were taken from the 3rd oldest leaf to the right of the main vein, using a metal bore hole, placed into pre-chilled 100μ L distilled water, with added ice crystals to aid freezing process. Sample was kept at 4 °C (on ice) for up to 30 min, then incubated at −1 °C for 1 hour 30 mins, then −5 °C for 1 hour 30 min. The samples were transferred back to 4 °C and 4.9 ml of distilled water added in 15 ml polypropylene tubes. This was then incubated shaking overnight at 4 °C. Samples were returned to room temperature and the electrical leakage (EC1) was measured using a conductivity meter (METTLER TOLEDO FE30) twice per sample. The total leakage (EC2) was determined after the samples were heated to 99 °C for 30 min. The ratio of EC1/EC2 × 100 was used to determine relative damage by cold stress (Elec_leakage). The experiment was performed on 3 biological replicates per genotype.

4.5. Photosynthesis analysis

In a separate experiment 94 genotypes of *B. napus* were sown in a randomised pattern for 10 biological replicates in propagation trays, 1 seed per well. Seeds were germinated in Levington Seed & Modular + Sand – F2S in a controlled environment growth room (21 °C/15 °C day/night with a 12hr day-night cycle). At 2 weeks old, half the replicates were subjected to heat stress in an environmental growth cabinet (35 °C/25 °C day/night with a 12hr day-night cycle) for 3 days, and then chlorophyll florescence was analysed in both control and heat stress plants.

The second true leaf was excised and arranged on a 2mm-thick damp filter paper, then placed between two glass plates (approx. 1 cm thick). Leaves were collected between 9–12am and dark adapted for 1hr before measurements following the Fv/Fm protocol of FLUORCAM 7 software (Photon System Instruments) (McAusland et al., 2019). Measurements of Fv/Fm were performed at room temperature (approx. 21 °C) to monitor any changes to the maximum quantum efficiency of PSII to evaluate the status of the photosynthetic machinery under heat treatment.

4.6. Phenotypic data transformation for association analysis

To calculate the recovery of seed weight after heat stress (Fig. 7), we calculated the difference between the effect of heat treatment (control seed weight/heat stress seed weight) on the main raceme and the whole plant. Each dataset, seed yield (number and weight), recovery of seed weight after temperature, were used for input into the AT pipeline (Fig. 7).

4.7. Association Transcriptomics (AT)

Genotype (SNP) and expression level datasets (Havlickova et al., 2018), available from York Knowledgebase (http://yorknowledgebase.info) were used and reduced to include only the 94 genotypes used within this study. The transcriptomics for SNP and gene expression marker analysis was conducted on the second true leaves from four replicate plants per genotype due to the large number of genes expressed at this timepoint. Sequence reads were mapped to the CDS gene model-based Brassica AC pan-transcriptome reference (He et al., 2015), which comprised 116,098 gene models for SNP scoring. 219,454 SNPs with maternal allele frequencies greater than 5 % were used for downstream analysis. Genome-Wide Association (GWA) and GEM mapping were done using the R-based GAGA pipeline (Nichols, 2022), which utilises GAPIT Version 3 (Lipka et al., 2012; Wang and Zhang, 2021). GAGA was run using the recently updated population structure (Fell et al., 2023) and *B. napus* Pan transcriptome version 11 (Havlickova et al., 2018). GWA analyses were done using a generalised linear model (GLM); Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK) (Huang et al., 2018), and Fixed and random model Circulating Probability Unification (FarmCPU) (Liu et al., 2016) to determine the optimal model. The best-fitting model was selected for final analysis by comparing QQ plots. Of these 94 genotypes a minimum of 76 were used for Association Transcriptomics as certain lines yielded unreliable data reducing the traits that could be analysed if included [Exclusion criteria, if <3 lines did not produce usable data in any of the 3 conditions (control, heat, cold) they were excluded from analysis for that specific trait].

GEM associations were determined by linear regression using Reads Per Kilobase of the transcript, per Million mapped reads (RPKM) to predict a quantitative outcome of the trait value. All markers with an average expression of <0.5 RPKM were removed before analysis resulting in 53,883 expression values for association analysis. The Pearson method was used to determine the correlation coefficient between expression and resistance phenotype for each GEM. The false discovery rates (FDR) for both GEM and GWA were determined using the Shiny implementation of the q-value R package (Storey, 2011).

METAL analysis was used to combine p-values across multiple GWA outputs for both seed recovery after heat treatment data and for seed yield under control conditions (seed number and seed weight) (Willer et al., 2010).

The level of linkage disequilibrium (LD) varies between and across chromosomes depending on the position and level of selection. To determine the specific level of LD at loci identified for the traits described above, we calculated the mean pairwise R2 for this marker compared to all markers on the chromosome using TASSEL Version 5.0 using the site by all analysis options (Bradbury et al., 2007). Markers were considered in LD when R2 > 0.2. The number of genes within LD was determined by comparison to *B. napus* Darmor v4.1 reference genome and gene function was inferred by comparison to Arabidopsis. Further homology between *B. oleracea* identified genes and *B. napus* was determined using BioMart and then conversion to *B. napus* Darmor v4.1 ID's from ensemble *B. napus* ID's (Smedley et al., 2009). ShinyGO 0.82 was used for enrichment analysis of the candidate genes (Ge et al., 2020).

4.8. Statistical analysis

Principal Components Analysis (PCA) was performed using princomp() package and visualised using ggplot in the R version 4.2.2 (Wickham, 2016; R Core Team, 2021), PCA was used to assess sources of variability among several variables measured from the *B. napus* genotypes phenotypic data across different stresses. The first two principal components explained 59.6 % of the total variance. A standard linear model was used to assess the difference between Stress and Cultivar for each variable. Stress and Cultivar were considered as factor variables and ANOVA was used to identify if Stress, Cultivar and/or their interaction explain significant amounts of variability in the data. The Least-Significant Difference (LSD) was used to test if the differences in means were significant from zero. The significance level used was 0.05. Transformations of the response were used to meet the assumption of Normality of the linear model.

Pearson's correlation was performed using cor() function to measure the linear relationship between two variables and corrplot() package to visualise in R version 4.2.2 (R Core Team, 2021; Wei and Simko, 2024). This was used to investigate the association between the different variables measured (e.g. between pod sterility and seed yield), in the different varieties (e.g. Winter/Spring) and among the different treatments (e.g. cold, control, heat).

Glossary

Association Transcriptomics (AT) - Is a genetic methodology that can be used to compare the genetic components of gene expression and the

genetic components of a trait to determine if an association is present between the two components

BBCH staging - The extended BBCH-scale is a system for a uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species. The entire developmental cycle of the plants is subdivided into ten clearly recognizable and distinguishable longer-lasting developmental phases. These principal growth stages are described using numbers from 0 to 9 in ascending order

BnASSYST diversity panel - A diverse population (530 member) of *Brassica napus* L. consisting primarily of winter biotypes was assembled and used in genome-wide association studies.

GWAS - A genome-wide association study (GWAS) is a research approach used to identify genomic variants that are statistically associated with a particular trait. The method involves surveying the genomes of many varieties, looking for genomic variants that occur more frequently in those with a specific trait compared to those without the trait.

Linkage Disequilibrium - is a term in population genetics referring to the association of genes, usually linked genes, in a population.

Principal Component Analysis (PCA) - is a dimensionality-reduction method used extensively in machine learning and statistics to transform a dataset consisting of potentially correlated variables into a set of linearly uncorrelated variables known as principal components.

QYmax - Maximum quantum yield (Qmax) in terms of Fv/Fm value, which indicates photochemical efficiency.

SNP - A single nucleotide polymorphism is a genomic variant at a single base position in the DNA.

CRediT authorship contribution statement

Alison C Tidy: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Laura Siles:** Writing – review & editing, Investigation, Formal analysis. **Catherine Jacott:** Writing – review & editing, Investigation, Formal analysis. **Rachel Wells:** Writing – review & editing, Funding acquisition. **Smita Kurup:** Writing – review & editing, Supervision, Funding acquisition. **Zoe A Wilson:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

There is no conflict of interest or competing interest associated with this research and submission.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2025.100957.

Data availability

Data will be made available on request.

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